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In silico identification and characterization of putative kuruma prawn (*Marsupenaeus japonicus*) allergens

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Abstract

Background: Prawn is one of the major sources of shellfish allergens, which will induce mild to severe allergic reactions.

Aim: The objective of this study was to predict putative allergens and its characteristics present in *Marsupenaeus japonicus* through an *in silico* approach. **Materials And Methods:** In this present study, *in silico* tools, RaptorX, Bepipred Linear Epitope and Parker Hydrophilicity Prediction method, and MHC2Pred were used to predict the putative and cross-reactive allergens, tertiary and secondary structures, B-cell linear epitopes, and T-cell epitopes, respectively. **Results:** As a result, eight putative and cross-reactive allergens including tropomyosin fast isoform, arginine kinase, sarcoplasmic calcium binding protein, myosin light chain, chymotrypsin like proteinase, superoxide dismutase, cathepsin B, and trypsin were predicted by analyzing the protein sequences of *M. japonicus*. Furthermore, five amino acids (Ala, Ser, Asn, Gly, and Lys) play a vital role in immunoglobulin E binding allergenic epitope. At least one of them was found in the predicted B-cell epitope for each of the predicted putative allergens. Moreover, the predicted T-cell epitopes were highly associated with human leukocyte antigen (HLA) DRB1*0101 and HLA-DRB1*0901 alleles while mediating T-cell immune responses.

Conclusion: These results can be utilized to contribute in peptide immunotherapy and reduce the allergic diseases related to shellfish.

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Full Text

Introduction

Food allergy is an adverse condition which occurs when an immune system counteracts with an allergen, stimulating immunoglobulin E (IgE) production that will cause hypersensitivity reactions.[1] Prawn is one of the major sources of

shellfish allergens, which will induce mild to severe allergic reactions.

In general, there were 16 allergens which have been identified in crustacean including hemocyanin, tropomyosin, arginine kinase, sarcoplasmic calcium-binding protein, troponin C, triosephosphate isomerase, fatty acid-binding protein, α -actin, smooth endoplasmic reticulum Ca²⁺ ATPase, enolase, glyceraldehyde-3-phosphate dehydrogenase, vitellogenin, ovarian peritrophin 1 precursor, b-actin, 14-3-3 protein, and myosin light chain.[2],[3],[4],[5],[6],[7],[8] Besides, these allergens were responsible for causing allergic disorders such as acute and chronic diseases include atopic dermatitis or eosinophilic gastroenteropathies.[9]

Although allergen-specific immunotherapy aims to treat food allergy, this approach can induce severe anaphylactic effects and may possibly cause sensitization toward new allergens. To overcome these side effects, different approaches have been designed including the usage of allergen-derived B-cell peptides, allergen-derived T-cell epitope containing peptides, and allergen-encoding DNA vaccination.[10],[11]

Hence, identification of putative allergens and its characteristics present in *Marsupenaeus japonicus* through an in silico approach are necessary in treating allergic diseases related to shellfish. *M. japonicus* is also known as kuruma prawn or Japanese tiger prawn. It is commonly found in Japan, Taiwan, China, and Korea.[12] Thus far, only two allergens (tropomyosin and sarcoplasmic calcium binding protein) have been identified in *M. japonicus*. [8],[13] It suggests that many allergens are still lie unidentified in this species.

Subjects and Methods

Protein sequence retrieval

A total number of 507 protein sequences were available from the Universal Protein Resource (UniProt) database (<http://www.uniprot.org/>) for this species. The 507 protein sequences of *M. japonicus* were retrieved from this database.

Prediction of allergenic protein by using prediction of allergenic proteins and mapping of immunoglobulin E epitopes (AlgPred)

The protein sequences of *M. japonicus* were entered individually into the AlgPred server (<http://www.imtech.res.in/raghava/algpred/submission.html>). This could help to scan for allergenic proteins based on the basis of IgE mapping, Support Vector Machine prediction on amino acid composition, hybrid approach, Multiple Em for Motif Elicitation, and Basic Local Alignment Search Tool search on allergen representative peptides. Consequently, a positive result obtained from any one of these five prediction approaches, the particular protein sequence would be assigned as a potentially allergenic protein. The potential allergenic protein sequences were saved to be used for further analysis.

Food and Agriculture Organization of the United Nations/World Health Organization allergenicity test

Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) allergenicity test was performed in Allermatch™ (<http://www.allermatch.org/allermatch.py/form>) by scanning the query protein sequence obtained from the AlgPred against all the available protein sequences in the UniProt and WHO and International Union of Immunological Societies Allergen Nomenclature Subcommittee databases. According to the FAO/WHO (2001) recommendations for identification of putative allergens, only sequences sharing the properties of more than 35% identity over a frame of 80 amino acids with known allergens or having an exact match of 6–8 contiguous amino acids would be highly considered as putative allergens. Those potential allergenic protein sequences which had satisfied the both criteria suggested by FAO/WHO would be used in the next procedure.

Prediction of putative allergen by using Allerdicator

The potential allergenic protein sequences obtained from the earlier allergenicity test were further analyzed with Allerdicator (<http://allerdicator.vbi.vt.edu/>). The protein sequences of the putative allergens were entered individually into the Allerdicator server. The server would generate allergen probability and categorize them into allergen and nonallergen. The result that showed allergen indicated the protein sequence was the putative allergen. The potential allergenic protein sequences were saved to be used for further analysis.

Protein tertiary structure prediction and validation

The potential allergenic protein sequences obtained from the Allerdicator were used as data input in the RaptorX structure prediction server (<http://raptorx.uchicago.edu/StructurePrediction/predict/>) to construct the three-dimensional (3D) models. Besides, the stereochemical quality of a 3D model generated by RaptorX server was evaluated with Verify 3D (http://services.mbi.ucla.edu/Verify_3D/). If the score obtained for a 3D model was – 1, indicated a bad score, whereas + 1 is generally considered as a good score.

Protein secondary structure prediction

The secondary structure of the putative allergen was generated together with the protein tertiary structure prediction in the previous procedure by using the protein sequence of interest as the data input in the RaptorX server (<http://raptorx.uchicago.edu/StructurePrediction/predict/>). The predicted secondary structures were divided into three parameters of states including helix, beta, and coil.

B-cell epitope prediction

The potential allergenic protein sequences obtained from the Allerdicator were used as the data input for antibody epitope prediction in Bepipred Linear Epitope and Parker Hydrophilicity Prediction method (<http://tools.immuneepitope.org/bcell/>). Bepipred Linear Epitope Prediction method helps to predict the location of linear B-cell epitopes using two methods including Hidden Markov Model and propensity scale. Two algorithms (Bepipred Linear Epitope and Parker Hydrophilicity Prediction method) have been utilized in determining of antigenicity and hydrophilicity in the prediction of B-cell epitopes of the putative allergens. The finalized consensus B-cell epitope result was obtained by comparing the predicted results of Bepipred and Parker Hydrophilicity.

T-cell epitope prediction

T-cell epitopes were predicted by using MHC2Pred (<http://www.imtech.res.in/raghava/mhc2pred/index.html>). MHC2Pred is used to predict the binding of peptides to major histocompatibility cell (MHC) Class II complex. Besides, the allele is also known as human leukocyte antigen (HLA) complex which corresponds to MHC Class II. The potential allergenic protein sequences obtained from the Allerdicator were used as the data input for T-cell epitope prediction. According to a study, HLA-DQB1*0201, HLA-DQB1*0302, HLA-DRB1*0101, HLA-DRB1*0301, HLA-DRB1*0401, and HLA-DRB1*0901 alleles were reported to be related to shellfish allergy.[14] HLA-DQB1*0201 and HLA-DQB1*0302 were selected and used to predict HLA-DQ-based T-cell epitope. HLA-DRB1*0101, HLA-DRB1*0301, HLA-DRB1*0401, and HLA-DRB1*0901 were selected and used to predict HLA-DR based T-cell epitope. Only the top hit of the peptide was chosen to be displayed in the result.

Results

According to the AlgPred analysis, 135 out of 507 proteins were considered as potential allergenic proteins present in *M. japonicus*. Based on the FAO/WHO allergenicity test, 48 proteins were predicted as putative allergens present in *M. japonicus*. As a result, a total of eight putative allergens were predicted present in *M. japonicus* by using AlgPred, Allermatch™, and Allerdicator [Table 1]. The predicted tertiary structures of the eight putative allergens as shown in [Figure 1]. Moreover, the predicted tertiary structures of the putative allergens were validated and evaluated by using verify 3D [Table 2]. It showed that most of the predicted 3D models of the eight putative allergens had scored 3D-1D scores which were near to 1, indicated good quality of 3D models except for tropomyosin fast isoform (0.0035). The percentage of secondary structure present in the predicted putative allergen was depicted in [Table 3]. The predicted secondary structures of all the eight putative allergens as shown in [Figure 2]. The finalized consensus result of the B-cell epitope chosen for each of the predicted putative allergens in *M. japonicus* was depicted in [Table 4]. Besides, the predicted B-cell epitope for each of the putative allergens was mapped onto the protein to visualize the position [Figure 3]. The top predicted peptide scores and sequences of T-cell epitopes bind on HLA-DR and HLA-DQ alleles as shown in [Table 5] and [Table 6], respectively. The first and second highest scores were recorded at 1.902 and 1.884, respectively, on HLA-DRB1*0101. {Figure 1}{Figure 2}{Figure 3}{Table 1}{Table 2}{Table 3}{Table 4}{Table 5}{Table 6}

Discussion

In silico protein analysis is a well-known technique which is used to assess the cross-reactivity and allergenicity of protein.

Sequences with known IgE epitopes often contribute to cross-reactivity among the allergens from different species.[1],[15] By comparing and matching the query protein sequence with known IgE epitopes, one can assess and predict the potential cross-reactivity allergens. If the query protein sequence is having unknown epitopes, one can identify the degree of similarities between the query protein and allergen sequence using homology searching method.[1],[15]

A total of eight putative allergens were predicted which includes tropomyosin fast isoform, arginine kinase, sarcoplasmic calcium-binding protein, myosin light chain, chymotrypsin-like proteinase, superoxide dismutase, cathepsin B, and trypsin. Tropomyosin fast isoform had shared 91.25% identity with black tiger prawn (*Penaeus monodon*), Pen m 1. Tropomyosin in *P. monodon* (A1KYZ2) had been documented to be an important allergen in the previous studies.[6],[13] Thus, it correlated well with the highly significant homology which would suggest that *M. japonicus* to be cross-reactive with *P. monodon*.

Arginine kinase also had shared the 91.25% identity with white leg shrimp (*Penaeus vannamei*), Lit v 2. According to García Orozco (2007),[3] arginine kinase had been reported as an allergen in *P. vannamei*. It was more likely that the arginine kinase of *M. japonicus* was cross-reacting with the arginine kinase reported in the *P. vannamei* due to sequence homology. Furthermore, sarcoplasmic calcium-binding protein also showed significant shared identity, 88.75% with black tiger prawn (*P. monodon*), Pen m 4. Sarcoplasmic calcium binding protein turned out to be an allergenic protein in *P. monodon*, white leg shrimp (*Litopenaeus vannamei*), crucifix crab (*Charybdis feriatus*), and brown shrimp (*Crangon crangon*).[2],[13],[16] Consequently, both of the sarcoplasmic calcium-binding proteins reported in this study would most probable allergens due to the occurrence of cross-reactivity in different organisms.

Myosin light chain is also one of the common allergens found in crustacean and it was ranked the seventh in sharing 83.75% identity with brown shrimp (*C. crangon*), Cra c 5. According to Bauermeister et al. (2008),[17] this protein was a novel allergen identified in *C. crangon*, as well as other types of crustacean species. There was a possibility of cross-reactive occurred between *M. japonicus* and *C. crangon*. In addition, chymotrypsin-like proteinase, superoxide dismutase, cathepsin B, and trypsin were found to be cross-react with other species such as rubber tree, kiwi, and mite, respectively.

According to the predicted tertiary structures of the eight putative allergens, the 3D models were validated and evaluated as good quality by RaptorX. The predicted tertiary models were validated by Verify 3D, with generated 3D-1D profiles. However, when this score factor was generated for 1c1gA, a template used by RaptorX server in protein modeling, a low score was also obtained. A low score was obtained for tropomyosin fast isoform due to the propensity of behaving as alpha helices and also the simplicity of its structure.[18]

As for secondary structure aspect, tropomyosin fast isoform had the propensity in behaving as alpha-helices, whereas arginine kinase had an almost similar propensity of behaving as alpha-helices (40%) and coils (46%) in the structure. Moreover, myosin light chain and sarcoplasmic calcium-binding protein were having fewer beta sheets in the structure. As for chymotrypsin-like proteinase, cathepsin B, and trypsin, they tend to have more coils in the structure which were at 57%, 58%, and 60%, respectively.

In this present study, the B-cell epitopes were predicted by the Bepipred found to be fitting suitably into the hydrophilic regions, which were subject to the Parker Hydrophilicity Prediction. More than half of the amino acid residues present in B-cell epitopes of tropomyosin fast isoform, arginine kinase, sarcoplasmic calcium-binding protein, and cathepsin B were hydrophilic. Thereby, these protein regions with greater distribution of hydrophilic residues were being exposed to the external surface which would most probably capable of inducing B-cell responses. It was strongly believed that large conserved solvent exposed residues on the surface of a particular protein were able to cross-reactive.[19]

In contrast, more than half of the amino acid residues present in B-cell epitopes of myosin light chain, superoxide dismutase, cathepsin B, and trypsin were less hydrophilic. A study reported that five amino acids including Ala, Ser, Asn, Gly, and Lys were playing a vital role in the IgE binding allergenic epitopes.[20] It can be observed that at least one of the five amino acids was found in the predicted B-cell epitopes for each of the putative allergens.

The prediction of T-cell epitopes of the putative allergens was playing a key role because they were responsible for inducing humoral and cell-mediated immune responses. From the T-cell epitope prediction results, the highest peptide scores for each of the putative allergens were reported in these two alleles such as HLA-DRB1*0101 and HLA-DRB1*0901. It was observed that the predicted T-cell epitopes were highly associated with the HLA-DRB1*0101 and HLA-DRB1*0901 while mediating T-cell immune responses. It was also reported that T-cell epitopes of *M. japonicus* had a considerably high association with HLA-DRB1*01401.

Different approaches have been designed to treat allergic diseases. In general, avoidance seems to be the best treatment for patients who suffered from a food allergy. Currently, T-cell peptide immunotherapy is used as a treatment for allergic diseases because IgE binding would never form, leading to no occurrence of allergic reactions. Besides, it can enable to

continue to preserve the activation of T-cells.

Conclusion

According to the in silico analysis results, the eight putative allergens, tertiary structures, secondary structures, B-cell linear epitopes, and T-cell epitopes of *M. japonicus* have successfully predicted in this research study. Thus, the predicted structures can be utilised in molecular comparison with other allergens. The predicted putative allergens, B-cell linear epitopes, and T-cell epitopes can help contributing in epitope-based peptide therapy which can provide immunological tolerance without triggering the IgE cross-linking effects and activation of inflammatory cell. Hence, further in vivo and in vitro analyses are required to validate and verify the potency of predicted epitopes bind to their respective HLA alleles.

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Conflicts of interest

There are no conflicts of interest.

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